TRANSLATION: JP 63146818 A

09/625,790

PTO: 2004-3340

Japanese Published Unexamined (Kokai) Patent Publication No. S63-146818; Publication Date: June 18, 1988; Application No. S62-181718; Application Date: July 20, 1987; Int. Cl.[4]: A61K 31/40 // C07D 207/12; Inventor(s): Ryuzo Ueno et al.; Applicant: Ueno Seiyaku Oyo Kenkyusho KK; Japanese Title: Uirusu-sei Shikkan Shochizai (Viral Disease Treating Agent)

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Specification

1. Title of Invention

Viral Disease Treating Agent

2. Claim(s)

1. A viral disease treating agent, characterized in that anisomycin or a salt thereof is used as an effective ingredient.

2. The viral disease treating agent, as disclosed in Claim 1, characterized by having a formulation of an external drug.

3. Detailed Description of the Invention

[Field of Industrial Application]

This invention pertains to viral disease treating agents.

[Technological Background and the History of the Invention]

The development of pharmaceuticals against viral diseases have significantly been delaying in comparison with those against bacterial diseases. Many of chemically synthesized substances demonstrate antiviral activities. As the side effect is severe, many

of those cannot be put to the practical use. Accordingly, an emergence of antiviral agents without having ant side effects is desired. As a result of searching antiviral activity containing substances among already known natural substances, the inventors have found that anisomycin that has an antiprotozoal activity and that is commercially available by a trademark name, Flagecidin, demonstrates an antiviral effect both DNA and RNA viruses, thereby attaining the invention.

[Prior Art]

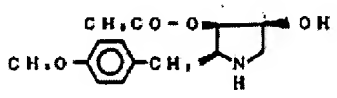
As for anisomycin producing methods, the following methods are known: a method for extracting anisomycin from a culture solution of streptomyces griscolus ATCC 11796 using methyl isobutyl ketone at pH 9 (US patent No. 2691618); a synthesizing method (Canadian Journal Of Chemistry Vol. 47, p. 2421; and Chemical And Pharmaceutical Bulletin Vol. 16, p. 2086 and Vol. 17 p. 1405). Anisomycin is also commercially available.

[Description of the Invention]

More specifically, the invention is a viral disease treating agent that contains anisomycin or a salt thereof as an effective ingredient.

The word "treating" in the application refers to various types of management including prevention and treatment.

Anisomycin is a substance having the following structural formula:



The chemical name is 3-acetoxy-4-hydroxy-2-(p-methoxybenzyl) pyrrolidine.

Anisomycin can be produced by either a fermentation or synthesis means. For example, anisomycin produced by the synthesis means can also be a mixture with substances having a different three-dimensional arrangement.

The following salts of anisomycin that are used by the invention are used: inorganic acid salts; and organic acid salts. The inorganic acid salts include hydrochloride, nitrate, sulfate, phosphate and the like, and the organic acid salts acetate, tartrate, toluene sulfonate and the like.

The dose of anisomycin or the salt is at an amount sufficient to generate an antiviral activity externally and internally. The dose is generally an amount in which an antiprotozoal activity occurs to about 1/10 of the amount, which is 0.25 to 100 $\mu$g/ml (preferably 0.5 to 10 $\mu$g/ml) or 0.2 to 200 mg/kg (preferably 0.5 to 25 mg/kg). The specific dose varies by the symptom, the age of the patient, the dosing means and the like. A topical administration (an external use) is used as a preferred dosing means as well as an injection, an inhalation and an oral administration.

The treating agent is dosed in a regular formulation after the effective ingredient has been mixed with a medicinal carrier suited for dosage. The following carriers are used: liquid paraffin; vaseline; silicon oil; aliphatic higher alcohols (acetyl alcohol, oleyl alcohol and the like); fatty acid esters (microcrystalline wax, isopropyl myristate and the like); lanolin; Plastibase (a mixture of liquid paraffin and polyethylene); polyethylene glycol; propylene glycol; glycerin; water; Arabic rubber; glucose; lactose; and starch. The following additives can also be added as needed: an emulsifier (fatty acid monoglyceride, sorbitan fatty acid ester, polyoxy ethylene lauryl ether or the like); a humectant (glycerin,

sorbitol or the like); a preservative (peroxy ethyl benzoate or the like); an antioxidant (BHA or the like); an pH adjuster (citric acid or the like); a suspender (CMC or the like); an isotonizer (sodium chloride or the like); and other regular additives.

The following formulations are also included in the treating agent produced using the aforementioned carriers and additives: an ointment type (oil or hydrophilic type); a lotion type; a liniment type; a cream type; a water type; an inhalant type; an eye drops type; a parenteral solution type; a tablet type and the like.

As for target viruses, DNA and RNA viruses are given. DNA viruses include the following types: herpesviridae such as simple herpes; poxviridae such as smallpox; and adenoviridae. RNA viruses include the following types: togaviridae such as Japanese encephalitis and rubella; pagamyxoviridae such as parotitis; orthomyxoviridae such as influenza; rhabdoviridae such as vesicular stomatitis; and retroviridae such as AIDS.

An acute toxicity ($Ld_{so}$) of anisomycin and the salt used for the invention is 167 mg/kg when they are given to rats by a venous injection means and 72 mg/kg when they are orally administrated.

[Working Examples]

The invention is described hereinbelow in detail based on the working examples so as to clarify the advantageous effect of the invention using the experiment.

Working Example 1

| | |
|---|---|
| (A) Anisomycin | 0.05 g |
| Isopropyl myristate | 5 g |

(B) Plastibase 94.95 g

After mixing the components of (A), the mixture is gradually added to (B) while it is agitated. The mixture is then uniformized to obtain an oil ointment.

Working Example 2

| | |
|---|---|
| (A) Anisomycin | 0.05 g |
| Polyethylene glycol (400) | 11.95 g |
| (B) Polyethylene glycol (400) | 12 g |
| Polyethylene glycol (4000) | 76 g |

After fusing (B) at 70°C, (A) mixed in advance is added to the fused (B) at 50°C. The mixture is then agitated until it is solidified. The solidified mixture is finally uniformized to obtain a hydrophilic ointment.

Experiment 1

(a) Preparation of a testing solution

Anisomycin is dissolved into dimethyl sulfoxido (DMSO) so as to become 200 μg/ml. The mixture is diluted into a 1/2 solution at five stages with DMSO. The ½ solution is added to a sample culture so as to be 0.5%. A sterilization is then applied using a membrane filter at 0.45 μm.

(b) Anti-DNA viral activity screening method

As for sample viruses, a KOS strain of herpes simplex virus I type and a SAVAG strain of herpes simplex virus II type are used. A vero cell is used as a host cell to be grown on a 5% calf blood serum added MEM culture. The screening is performed by

using a regular method. More specifically, the vero cell is inoculated in a short testing tube. When the cell forms a single layer, the sample viral liquid at 0.1 ml (m.o.i. = 0.2) is injected. After leaving the cell for 1 hour, the virus is absorbed in the cell. After an unabsorbed virus has been removed by a suctioning means, the cell is cultured for 20 hours adding the chemical agent containing MEM culture. After the completion of the culturing, a freeze-melt operation between -80°C and 37°C is repeated three times. Following this, the yield of the virus is assayed on a testing solution diluted by 1/10 at 5 stages by using a plaque method.

[c] Anti-RNA viral activity screening method

As for a sample virus, a coxackie virus $B_4$ is used. A BSC-40 cell is used as a host cell to be grown on a 10% calf blood serum added new MEM culture. The screening is performed by using a regular method. More specifically, the BSC-40 cell is spread in a Petri dish at $\phi$ 3.5 cm. When the cell forms a single layer, the sample virus at 0.1 ml (m.o.i. = 0.01) is injected. After leaving the cell for 1 hour, the virus is absorbed in the cell. The cell is cultured for 18 hours adding the chemical agent containing MEM culture at 2 ml each. After the completion of the culturing, the cell is peeled using a purusukon [Translator's Note: the word is not located in any dictionaries] bar and then transferred to a serum tube. A freeze-melt operation between -80°C and 37°C is repeated three times. Following this, the yield of the virus is assayed on a testing solution diluted by 1/10 at 5 stages by using the plaque method.

(d) Result

The results are indicated in the following tables.

1. KOS strain of herpes simplex virus (HSV) I type

| Concentration of anisomycin (μg/ml) | Yield of the virus PFU/ml ($x10^7$) | Inhibition ratio (%) |
|---|---|---|
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| | | |

2. SAVAGE strain of herpes simplex virus (HSV) II type

| Concentration of anisomycin (μg/ml) | Yield of the virus PFU/ml ($x10^6$) | Inhibition ratio (%) |
|---|---|---|
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| | | |

3. Coxackie virus $B_4$

| Concentration of anisomycin (μg/ml) | Yield of the virus PFU/ml ($x10^5$) | Inhibition ratio (%) |
|---|---|---|
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| | | |

As a result of the above experiment, it is found that anisomycin demonstrates an antiviral activity on both DNA and RNA viruses.

U.S. Patent and Trademark Office
Translations Branch
5/12/04
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